Effect of Vitamin C on Cyproterone Acetate Induced Genotoxic Damage in Mice

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Abstract: The genotoxicity study of cyproterone acetate was carried out in mouse bone marrow cells using Sister Chromatid Exchanges (SCEs) and Chromosomal Aberrations (CAs) as a parameter. The effect of cyproterone acetate was studied at 6.25, 12.50 and 25.00 mg kg^{-1} of body weight and was found to be genotoxic at 12.50 and 25.00 mg kg^{-1} of body weight. Further the effect of vitamin C on 25.00 mg kg^{-1} of body weight of cyproterone acetate was also studied. Three doses of vitamin C i.e. 20, 40 and 60 mg kg^{-1} of body weight were given along with 25.00 mg kg^{-1} of body weight of cyproterone acetate. Treatments along with vitamin C results in a significant decrease in CAs and SCEs suggesting an antigenotoxic role of vitamin C against cyproterone acetate induced genotoxic damage in mice bone marrow cells.

Key words: Cyproterone acetate, vitamin c, chromosomal aberrations, sister chromatid exchanges, mice bone marrow cells

INTRODUCTION

Synthetic progestins are widely used as oral contraceptives in addition to their use in the treatment of various menstrual disorders, various types of cancers and in hormonal replacement therapy. Cyproterone acetate is a potent steroidal antiandrogen with progestational activity. It is used alone or in combination with ethynylestradiol or estradiol valerate in the treatment of women suffering from disorders associated with androgenization, e.g. age or hirsuitism. Cyproterone acetate competes with dihydrotestosterone for the androgen receptors and inhibits translocation of the hormone receptor complex into the cell nucleus[1]. Cyproterone acetate has been shown to induce DNA repair synthesis in rat and human hepatocytes[2-4] and to form adducts in rat liver cells[5]. Further findings shows that cyproterone acetate is not only genotoxic but is also a tumour initiating agent in the liver of female rats[6,7]. It induced chromosomal aberrations in V79 cells[8] and in human lymphocytes *in vitro*[9] and sister chromatid exchanges in human lymphocytes *in vitro*[9]. In the present study we decided to see the effect of cyproterone acetate at three different doses on Sister Chromatid Exchanges (SCEs) and Chromosomal Aberrations (CAs) frequencies in mouse bone marrow cells and further the effect of different doses of vitamin C on one of the genotoxic dose of cyproterone acetate was also observed.

MATERIALS AND METHODS

Chemicals: Cyproterone acetate (CAS No: 427-51-0, Sigma); Dimethylsulphoxide (0.1 mL/animal, E. Merck, India); Colchicine (6.0 mg kg^{-1} body weight); Hoechst 33258 stain (0.05% w/v, Sigma); 3% and 7% Giemsa solution in phosphate buffer (pH 6.8, E. Merck, India); N-methyl-N'-nitro-N-nitrosoguanidine (1.2×10^4 µg kg^{-1} body weight); 5-Bromo-2-deoxyuridine (1.6 g kg^{-1} body weight); vitamin C (CAS No: 50-81-7, Central Drug House, India).

Animals: Swiss albino female mice (*Mus musculus* L.) 25-30 g, 10-12 weeks old were procured from Lucknow (U.P.), India and grouped in different cages (5 animals/group) and kept in propylene cages at a mean temperature of 25°C.

Sister Chromatid Exchange (SCE) analysis: The fluorescent plus Giemsa Technique[10] were followed for Sister Chromatid Exchange (SCE) analysis. 5-Bromo-2-deoxyuridine in tablet form (BrdU; 1.6 g kg^{-1} body weight) was implanted subcutaneously in the neck region of each mouse under mild anaesthesia and 30 min later, cyproterone acetate was injected intraperitoneally (i.p.) at 6.25, 12.5 and 25.0 mg kg^{-1} body weight to different group of animals. According to the LD50 obtained with the method of Lorke[11], which gave a result of 100 mg kg^{-1} of

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body weight, the highest tested corresponds to ¼ of this parameter. About 0.1 mL of dimethylsulfoxide (DMSO) and 1.2×10^4 µg kg^{-1} body weight of N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) were taken negative and positive controls, respectively. After 21 h, the animals received an intraperitoneal (i.p.) injection of colchicine (6.0 mg kg^{-1} body weight) and 3 h later, the bone marrow of both femurs were obtained in KCl 0.075 M at 37°C keeping the cells for 30 min at the same temperature. The supernatant was removed by centrifugation and 5 mL of fixative (methanol: glacial acetic acid; 3:1) was added. The fixative was removed and the procedure was repeated twice. Slides were stained for 20 min in a 0.05% (w/v) Hoechst 33258 solution, rinsed with tap water and placed under a UV lamp, for 90 min, covered with 'Sorensen's buffer, pH 6.8 and stained with 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min. The SCE average was taken from an analysis of the metaphases during the second cycle of divisions. About 60 second division mitoses per mouse were scored to determine the frequency of SCEs.

Chromosomal Aberration (CA) analysis: For the analysis of chromosomal aberration the tested and control doses were same as described for the SCE analysis. Cyproterone acetate was injected intraperitoneally (i.p.) at 6.25, 12.5 and 25.0 mg kg^{-1} body weight, to different group of animals. After 21 h, the animals were injected i.p. with colchicine (6.0 mg kg^{-1} body weight) 2 h before sacrifice. Bone marrow preparations for the analysis of chromosomal aberrations in metaphases cells were

obtained by the technique of Yosida and Amano[12]. Slides were stained with 7% Giemsa stain in phosphate buffer (pH 6.8). Five animals were taken for each treatment and 100 well spread metaphases were analysed per animal.

Treatment of cyproterone acetate along with vitamin C: About, 25.0 mg kg^{-1} of body weight of cyproterone acetate was given along with 20, 40 and 60 mg kg^{-1} body weight of vitamin C intraperitoneally to different groups of animals. The doses of Vitamin C falls within the dose range used in humans[13]. Vitamin C treatment was also given separately to different groups of animals. Analysis of chromosomal aberrations and sister chromatid exchanges were done similarly as described earlier in the text.

Statistical analysis: Student "t" test was used for the analysis of chromosomal aberrations and sister chromatid exchanges. The level of significance was tested from standard statistical table of Fisher and Yates[14].

RESULTS

In sister chromatid exchange analysis, a clear dose dependent increase in SCEs/cell was observed when treated with cyproterone acetate treatment was given alone Table 1. SCEs/cell were significantly increased at 12.50 and 25.0 mg kg^{-1} of body weight. When 25.0 mg kg^{-1} body weight of cyproterone acetate treatment was given along with different doses of vitamin C i.e., 20, 40 and 60 mg kg^{-1} body weight, a clear significant dose dependent decrease in SCEs/cell was observed Table 2.

Table 1: Frequency of sister chromatid exchanges induced by cyproterone acetate in mice bone marrow

Treatment	No. of metaphases analysed	Mean SCEs/metaphase±SE	Range
Cyproterone acetate (mg kg^{-1} body weight)			
6.25	300	3.02±0.27	1-6
12.25	300	10.34±0.73[a]	2-12
25.0	300	12.33±0.81[a]	2-13
Untreated	300	2.36±0.18	0-5
Positive control (MNNG, 1.2×10^4 µg kg^{-1} body weight)	300	13.41±0.93[a]	3-15
Negative control (DMSO, 0.1 mL/animal)	300	2.01±0.12	0-5
BrdU (1.6 g kg^{-1} body weight)	300	2.84±0.23	0-5

[a]Significant with respect to untreated (p<0.01). DMSO: Dimethylsulphoxide; BrdU: 5-Bromo-2-deoxyuridine. MNNG: N-methyl-N′-nitro-N-nitrosoguanidine

Table 2: Frequency of sister chromatid exchanges induced by cyproterone acetate and vitamin C treatment

Treatment	No. of metaphases analysed	Mean SCEs/metaphase±SE	Range
Cyproterone acetate (mg kg^{-1} body weight)			
25.0	300	13.22±0.89[a]	3-15
Cyproterone acetate (mg kg^{-1} body weight) +			
Vitamin C (mg kg^{-1} body weight)			
25 + 20	300	9.41±0.70[b]	2-11
25 + 40	300	8.53±0.67[b]	2-10
25 + 60	300	7.16±0.61[b]	2-9
Vitamin C (mg kg^{-1} body weight)			
20	300	2.41±0.20	0-5
40	300	2.73±0.22	0-5
60	300	2.89±0.25	0-5
Untreated	300	2.13±0.14	0-5

[a]Significant with respect to untreated (p<0.01). [b]Significant with respect to cyproterone acetate (p<0.05)

Table 3: Mean percentage of chromosomal aberrations in mouse bone marrow cells after cyproterone acetate

Treatment	Abnormal metaphases without gaps						Chromosomal aberrations				
	Mean %	± SE	Gaps		Fragments and/or breaks		Robertsonian translocations		Polyploidy	
			Number	%	Number	%	Number	%	Number	%
Cyproterone acetate (mg kg^{-1} body weight)										
6.25	10	2.0±0.6	6	1.2	8	1.6	-	-	2	0.4
12.50	45	9.0±1.2[a]	19	3.8	35	7.0	4	0.8	6	1.2
25.0	75	15.0±1.5[a]	31	6.2	53	10.6	9	1.8	13	2.6
Untreated	8	1.6±0.5	6	1.2	7	1.4	-	-	1	0.2
Positive control MNNG (1.2x10^4 µg kg^{-1} body weight)	130	26.0±1.9[a]	31	6.2	86	17.2	23	4.6	21	4.2
Negative control (DMSO, 0.1 mL/animal)	7	1.4±0.5	5	1.0	6	1.2	-	-	1	0.2

One hundred cells were analyzed per animal, for a total of 500 cells per treatment. [a]Significant with respect to untreated (p<0.01). DMSO: Dimethylsulphoxide; S.E: Standard error. MNNG: N-methyl-N′-nitro-N-nitrosoguanidine

Table 4:Mean percentage of chromosomal aberrations in mouse bone marrow cells after cyproterone acetate and Vitamin C treatment

Treatment	Abnormal metaphases without gaps						Chromosomal aberrations				
	Mean %	± SE	Gaps		Fragments and/or breaks		Robertsonian translocations		Polyploidy	
			Number	%	Number	%	Number	%	Number	%
Cyproterone acetate (mg kg^{-1} body weight)										
25	77	15.4±1.6[a]	32	6.4	54	10.4	8	1.6	15	3.0
Cyproterone acetate (mg kg^{-1} body weight) + Vitamin C (mg kg^{-1} body weight)										
25+20	45	9.0±1.2[b]	17	3.4	34	6.8	4	0.8	7	1.4
25+40	39	7.8±1.1[b]	14	2.8	31	6.2	2	0.4	6	1.2
25+60	27	5.4±1.0[b]	13	2.6	23	4.6	-	-	4	0.8
Vitamin C (mg kg^{-1} body weight)										
20	9	1.8±0.5	7	1.4	8	1.6	-	-	1	0.2
40	11	2.2±0.6	8	1.6	10	2.0	-	-	1	0.2
60	13	2.6±0.7	8	1.6	11	2.2	-	-	2	0.4
Untreated	7	1.4±0.5	5	1.0	6	1.2	-	-	1	0.2

One hundred cells were analyzed per animal for a total of 500 cells per treatment. [a]Significant with respect to untreated (p<0.01). [b]Significant with respect to cyproterone acetate (p<0.05) S.E: Standard error

In chromosomal aberration analysis, a dose dependent increase was observed in number of abnormal cells. However, a significant increase was observed at 12.25 and 25.0 mg kg^{-1} body weight of cyproterone acetate Table 3. The treatment of 25.0 mg kg^{-1} body weight of cyproterone acetate along with 20, 40 and 60 mg kg^{-1} body weight of Vitamin C results in a significant decrease in the number of abnormal cells Table 4.

DISCUSSION

In the present investigation cyproterone acetate increases Chromosomal Aberrations (CAs) and Sister Chromatid Exchanges (SCEs) frequencies at two of three tested doses. Similar observations on the DNA damaging properties of steroids as evident from chromosomal damage, induction of SCEs[15-21] and formation of endogenous DNA adducts and certain neoplastic changes have also been reported earlier[22]. The use of synthetic progestins cannot be completely eliminated in oral contraceptives, but the genotoxic damage caused by them can be reduced by the use of antioxidants[23-24] and natural plant products[25-27]. Cyproterone acetate has been shown to induced genotoxic damage in human lymphocytes by generating free radicals[9].

Vitamins act as antioxidants and free radical scavengers, thereby acting as anticarcinogenic, anticlastogenic and antimutagenic agents. Of these vitamin C and α-tocopherol are among the best known antioxidants used in *in vivo* animal models[28]. Vitamin C is an antioxidant, it possess substantial nucleophilic character and it has been suggested that, ascorbate might protect against electrophilic attack on cellular DNA by intercepting reactive agents[29] or that ascorbyl anion radical, with the high extent of unpaired electron delocalization, is responsible for the scavenging of free radicals[30-31]. However, ascorbic acid has been shown to be cytotoxic at higher concentrations, but the selected

doses lie within the range of humans[32]. Our study shows induction of sister chromatid exchanges and chromosomal aberrations at significant level by two out of the three tested doses of cyproterone acetate. Most of the chromosomal aberrations observed in cells are lethal, but there are many corresponding aberrations that are viable and can cause genetic effects, either somatic or inherited. Vitamin C treatment results in the reduction of genotoxic damage induced by cyproterone acetate suggesting a protective role of vitamin C against the genotoxic damage.

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